Mechanism by Which Mcl-1 Regulates Cancer-Specific Apoptosis Triggered by mda-7/IL-24, an IL-10–Related Cytokine

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Abstract
Melanoma differentiation-associated gene-7/interleukin-24 (mda-7/IL-24), a cytokine belonging to the IL-10 family, selectively induces apoptosis in cancer cells without harming normal cells by promoting an endoplasmic reticulum (ER) stress response. The precise molecular mechanism by which the ER stress response culminates in cell death requires further clarification. The present study shows that in prostate carcinoma cells, the mda-7/IL-24–induced ER stress response causes apoptosis by translational inhibition of the antiapoptotic protein myeloid cell leukemia-1 (Mcl-1). Forced expression of Mcl-1 blocked mda-7/IL-24 lethality, whereas RNA interference or gene knockout of Mcl-1 markedly sensitized transformed cells to mda-7/IL-24. Mcl-1 downregulation by mda-7/IL-24 relieved its association with the proapoptotic protein Bak, causing oligomerization of Bak and leading to cell death. These observations show the profound role of the Bcl-2 protein family member Mcl-1 in regulating cancer-specific apoptosis induced by this cytokine. Thus, our studies provide further insights into the molecular mechanism of ER stress–induced cancer-selective apoptosis by mda-7/IL-24. As Mcl-1 is overexpressed in the majority of prostate cancers, mda-7/IL-24 might provide an effective therapeutic for this disease. Cancer Res; 70(12): 5034–45. ©2010 AACR.

Introduction
Prostate cancer is the most common cancer and the second leading cause of cancer-related death in men in the United States (1). It is estimated that 1 man in 6 will be diagnosed with prostate cancer during his lifetime. Thus, it is imperative to develop more effective therapeutic approaches for late-stage prostate cancer (2).

Using subtraction hybridization combined with induction of cancer cell terminal differentiation, our laboratory cloned melanoma differentiation-associated gene-7/interleukin-24 (mda-7/IL-24; ref. 3), a novel member of the IL-10–related cytokine gene family (4, 5). Subsequent studies documented that mda-7/IL-24 has almost ubiquitous antitumor properties in vitro and in vivo, leading to its entry into the clinic, where its safety and clinical efficacy, when administered by adenovirus (Ad.mda-7; INGN 241), was observed in a phase I clinical trial in humans with advanced carcinomas and melanomas (6–9). Its mode of action involves preferential induction of apoptosis in cancer cells while exerting no discernible toxic effects toward normal cells (10–16) by eliciting potent “antitumor bystander activity” as a consequence of autocrine secretion (14, 17).

On ectopic expression of mda-7/IL-24 by an adenovirus, the MDA-7/IL-24 protein interacts with the endoplasmic reticulum (ER) chaperone protein Bip/GRP78 and initiates a cascade of “unfolded protein response” (UPR) events in tumor cells that culminate in apoptosis (18–20). Several well-known apoptosis-related molecules play critical roles in UPR-mediated apoptosis, among them myeloid cell leukemia-1 (Mcl-1), and have been reported to be downregulated/inactivated (21, 22). Mcl-1 plays a pivotal role in cancer cell survival, including that of prostate cancer cells, suggesting involvement of this gene in prostate carcinogenesis (23). Depletion of Mcl-1 using antisense oligonucleotides rapidly triggers apoptosis in leukemia cells (24). On the other hand, overexpression of Mcl-1 protects cells from apoptosis induced by a variety of stress-promoting agents, including UV, etoposide, staurosporine, and actinomycin D (25, 26).

In this study, we report that in prostate cancer cells, mda-7/IL-24 potently induces apoptosis in association with a pronounced reduction in Mcl-1 expression followed by Bak activation. Moreover, prevention of mda-7/IL-24–mediated Mcl-1 downregulation by ectopic expression of Mcl-1...
markedly diminishes mda-7/IL-24–induced mitochondrial injury and apoptosis. Finally, the present results indicate that mda-7/IL-24 downregulates Mcl-1 expression through inhibition of translation, rather than through a transcriptional mechanism. The present study highlights Mcl-1 as a potential target for therapeutic intervention in prostate cancer and identifies mda-7/IL-24 as a potent translational regulator of this critical cancer survival molecule.

Materials and Methods

Cells and stable clones
M12 and M2182 (progressed prostate cancer cells obtained from Dr. Joy Ware, VCU School of Medicine, Richmond, VA), DU-145, PC-3, P69, and mouse embryonic fibroblasts (MEF) were cultured as described (27, 28). DU-145 ectopically expressing Mcl-1 clones were generated as described previously (27).

Assessment of cell death and apoptosis
Aptotic cells were identified and quantified by Annexin V–fluorescein isothiocyanate staining (16), and cell death was quantified by trypan blue dye exclusion assays (29).

Quantitative real-time PCR
DU-145 cells were infected with Ad.vec or Ad.mda-7 for the indicated period, after which they were lysed and total RNA was extracted using the RNeasy mini kit (Qiagen). Quantitative real-time PCR analysis for Mcl-1 was performed as previously described (30).

Transient transfections and reporter gene assays, immunoprecipitation, and immunoblotting
Transfections used Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol, and the plasmid constructs have been described previously (30, 31). Reporter gene assays for Mcl-1 were carried out as previously described (31). Immunoprecipitation and immunoblotting were performed as described previously (31). The primary antibodies used in this study are Mcl-1, ubiquitin, BiP/GRP78, Bak, phosphorylated protein kinase R–like ER kinase (phospho-PERK; Santa Cruz Biotechnology, Inc.); poly(ADP-ribose) polymerase (PARP; Biomol Research Laboratories); Bcl-xL, total and phospho-ERK1/2 (Thr-202/Tyr-204), phospho-4EBP1 (Ser65), phospho-eIF4E (Ser209), phospho-eIF4G (Ser1108), total and phospho-ERK1/2 (Thr-202/Tyr-204), phospho-4EBP1 (Ser65), phospho-eIF4E (Ser209), phospho-eIF4G (Ser1108), total and phospho-p38 (Thr180/Tyr182), total and phospho-Akt (Thr183/Tyr185), total and phospho-eIF4G, total and phospho-eIF4E, phospho-eIF2 (Cell Signaling Technology); MDA-7/IL-24 (Gen Hunter Corporation); anti-eIF2α (Upstate Biotechnology); GRP94 (1:1,000, Sigma); and Bak Ab-1 (Calbiochem).

Mcl-1 protein stability and isolation of polysomes
DU-145 cells were infected with Ad.vec or Ad.mda-7 at 100 plaque-forming units (pfu)/cell for 16 hours, and pulse chase was performed, as previously described (30), for the indicated times. Polysomes were purified (32) from DU-145 cells 48 hours following treatment with Ad.vec or Ad.mda-7.

Human prostate cancer xenografts in athymic nude mice and immunohistochemistry
DU-Mcl-1-8 and pcDNA3.1 cells (3 × 10⁶) were injected s.c. in 100 μL of PBS in the left flank of male athymic nude mice, and xenograft studies were conducted as described previously (33). Formalin-fixed and paraffin-embedded specimens were sectioned 3- to 4-μm thick, and immunohistochemistry was done as described previously (33).

Detection of Bak and Bax activation
For Bak oligomerization studies, DU-Mcl-1-8 and pcDNA3.1-6 cells were treated with the indicated titer of Advec or Ad.mda-7 for 48 hours, and Bak oligomerization studies were performed (34). Bak and Bax activation were detected by flow cytometry (35).

Proteasome activity assays
DU-145 cells were infected with Ad.vec or Ad.mda-7, and lysates were collected after 12, 24, 48, and 72 hours. Proteasomal activity was measured using the 20S Proteasome Activity Assay Kit (Millipore Corporation) according to the manufacturer’s protocol.

Statistical analysis
Data are represented as the mean ± SD and analyzed for statistical significance using one-way ANOVA followed by Newman-Keuls test as a post hoc test. A P value of <0.05 was considered significant.

Results

Mcl-1 is overexpressed in human prostate carcinogenesis
We first established elevated expression of Mcl-1 in prostate cancer cell lines and patient-derived samples. P69 is a normal human prostate epithelial cell line immortalized by SV40 T/t antigen, M2182 is a tumorigenic but nonmetastatic P69 variant, and M12 is a tumorigenic and metastatic variant of P69, whereas PC-3 and DU-145 cells are highly aggressive tumorigenic patient-derived prostate carcinoma cell lines. Low-level Mcl-1 expression was detected in P69 cells, whereas robust expression of Mcl-1 was detected in M12, DU-145, and PC-3 cells (Fig. 1A). In nonmetastatic M2182 cells, a lower level of Mcl-1 expression was evident compared with M12, DU-145, and PC-3 cells. These findings were extended using two tissue microarrays (IMT-01291 and IMT-01286) that were immunostained using anti Mcl-1 antibody. Very little or no Mcl-1 immunostaining was detected in the 17 normal prostate biopsy samples (Supplementary Table S1), whereas significant Mcl-1 staining was observed in prostate cancer samples (Fig. 1B–F). Among the 100 PC samples, 87 showed variable levels of Mcl-1 expression.

Infection of DU-145 cells with Ad.mda-7 results in decreased Mcl-1 protein and induction of ER stress
Infection of DU-145 cells with Ad.mda-7 resulted in a time- and dose-dependent decrease in Mcl-1 protein levels, which correlated with increased expression of MDA-7/IL-24.
(Fig. 2A and B) and induction of apoptosis. At 48 hours, when Mcl-1 was extinguished, 43% of cells were apoptotic as detected by Annexin V–FITC/PI staining and PARP cleavage (Fig. 2A). Additionally, in DU-145 cells, the levels of BiP/GRP78, GRP94, activation of PERK, and eukaryotic initiation factor 2-α (p-eIF2α [Ser51]) were increased in a time-dependent manner, which correlated with decreased Mcl-1 protein levels (Fig. 2A). On the other hand, infection of DU-145 cells with Ad.GFP or Ad.LUC (100 pfu/cell) did not result in downregulation of Mcl-1 or induction of apoptosis (data not shown). In DU-145 cells, transient expression of a dominant-negative PERK or dominant-negative eIF2α protected from Ad.mda-7–mediated downregulation of Mcl-1 as well as apoptosis (Fig. 2C and D). In PC-3 cells, infection with a tropism-modified adenovirus Ad5/3.mda-7 (33) resulted in significant accumulation of MDA-7/IL-24 protein with a concomitant decrease in Mcl-1 and induction of apoptosis (Fig. 2E). In P69 normal immortal human prostate epithelial cells, Mcl-1 downregulation was not observed on infection with Ad.mda-7, and apoptosis was not induced (Fig. 2F).

Enforced expression of Mcl-1 substantially blocks Ad.mda-7–mediated apoptosis in human prostate cancer cells

To investigate whether Mcl-1 downregulation plays a functional role in Ad.mda-7–mediated lethality, DU-145 cells were transiently transfected with a Mcl-1 expression construct. Infection with Ad.mda-7 resulted in a marked decrease in Mcl-1 protein level in control cells; however, levels of Mcl-1 protein in Mcl-1-overexpressing cells remained higher than those of untreated empty vector controls. Notably, a significant reduction in Ad.mda-7–mediated PARP cleavage was observed in Mcl-1–transfected cells at 48 hours postinfection (Fig. 3A). Parallel studies were done in DU-145 cells stably transfected with a Mcl-1 construct and overexpressing Mcl-1, in which nearly identical protection (\( P < 0.05 \) versus DU-pcDNA3.1) from Ad.mda-7–mediated lethality was noted (Fig. 3B and C). Clones 8 and 20 (DU-Mcl-1-8 and DU-Mcl-1-20) stably overexpressed Mcl-1 at higher levels compared with DU-145 cells (DU-pcDNA3.1) stably transfected with empty pcDNA3.1 vector, which was used as a control. DU-Mcl-1-8, which expressed elevated Mcl-1 compared with the DU-Mcl-1-20 clone (Fig. 3B), was used for further experiments.

To determine if the in vitro resistance of DU-Mcl-1-8 to Ad.mda-7 infection also translated to the in vivo setting, DU-Mcl-1-8 and DU-pcDNA3.1 tumors were established in the left flanks of athymic nude mice. After palpable tumors of \( \sim 75 \text{ mm}^3 \) developed, in \( \sim 7 \) to 10 days, the animals received seven intratumoral injections over a 4-week period, with \( 4.5 \times 10^6 \) pfu of Ad.vec or Ad.mda-7. In the DU-Mcl-1-8 xenograft model, a modest antitumor growth-inhibitory effect was seen with injection of Ad.mda-7 as evident from measurement of both tumor size and weight (Fig. 3D and E). In contrast, Ad.mda-7 exhibited a dramatic inhibition in growth of the DU-pcDNA3.1 tumors. In both the DU-Mcl-1-8 and pcDNA3.1 xenografts, infection with Ad.mda-7 generated a significant amount of MDA-7/IL-24 protein; however, increased terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) staining and decreased expression of Ki-67 were observed only in DU-pcDNA3.1 xenografts. In DU-Mcl-1-8 xenografts, no significant increase in TUNEL staining and no decrease in Ki-67 staining were observed (Fig. 3F).

Mcl-1 downregulation promotes activation of Bak

One of the mechanisms by which Mcl-1 opposes apoptosis is by binding and sequestering the proapoptotic protein Bak (35). Therefore, we investigated Bak activation following infection with Ad.vec or Ad.mda-7 in the DU-Mcl-1-8 and DU-pcDNA3.1 clones. Total Bak levels were unaffected in either clone by infection with Ad.mda-7 (Fig. 4A). Immunoprecipitation and flow cytometric analysis using an activated form of anti-Bak antibody (clone Ab-1) revealed that in

![Figure 1](cancerres.aacrjournals.org)
Figure 2. Forced expression of Mcl-1 blocks mda-7/IL-24–mediated apoptosis in DU-145 cells in vitro and in vivo. A, DU-145 cells were infected with 100 pfu/cell of Ad.vec or Ad.mda-7, protein lysates were prepared at the specified time points, and expression profiles of the indicated proteins were determined by Western blotting. B, DU-145 cells were infected with the indicated pfu/cell of Ad.vec or Ad.mda-7 for 48 h, and expression of Mcl-1 and MDA-7/IL-24 and activation of eIF2α were determined by Western blotting. C, DU-145 cells were transfected with a dominant-negative PERK (PERK DN) or control vector and then infected with the indicated pfu/cell of either Ad.vec or Ad.mda-7 for 48 h. Expression of the indicated proteins was determined by Western blotting. D, DU-145 cells were transfected with an eIF2αDN or control vector and then infected with indicated pfu/cell of either Ad.vec or Ad.mda-7 for 48 h. Expression of the indicated proteins was determined by Western blotting. E, PC-3 prostate cancer cells were infected with the indicated pfu/cell of Ad.5-vec, Ad.5/3-vec, Ad.5/3-mda-7, or Ad.5/3-mda-7, and expression of Mcl-1 and MDA-7/IL-24 was determined by Western blotting. F, normal prostate epithelial cells (P69) were infected with the indicated pfu/cell of Ad.vec or Ad.mda-7, and expression of Mcl-1 and MDA-7/IL-24 was determined by Western blotting. Percentage of apoptosis in A to F was determined by Annexin V–fluorescein isothiocyanate/propidium iodide (Annexin V–FITC/PI) staining. Bars, SD (n = 3).
DU-pcDNA3.1 cells, activated Bak appeared at 48 hours after Ad.mda-7 infection. In contrast, in DU-Mcl-1-8 cells, significantly less Bak activation was observed (Fig. 4A and B). Correlating with these data, 48 hours after infection with Ad.mda-7, Bak oligomers were readily observed in DU-pcDNA3.1 cells but not in DU-Mcl-1-8 cells (Fig. 4C). Similarly, the faster migrating intramolecular cross-linked species of Bak (inactive form of Bak) was only observed in Ad.vec-infected DU-pcDNA3.1 cells and Ad.vec- or Ad.mda-7–infected DU-Mcl-1-8 cells, but not in Ad.mda-7–infected DU-pcDNA3.1 cells. Further, immunoprecipitation assays using anti-Bak antibody revealed that 24 hours after infection with Ad.mda-7, DU-Mcl-1-8 cells contained noticeable amounts of Mcl-1 associated with Bak. In contrast, no interaction between Bak and Mcl-1 was evident in DU-pcDNA3.1 cells following Ad.mda-7 infection, presumably due to Mcl-1 downregulation (Fig. 4D). Moreover, in DU-pcDNA3.1 cells, the pan-caspase inhibitor z-VAD-FMK was ineffective in preventing Mcl-1 downregulation caused by Ad.mda-7 infection. Furthermore, in DU-145, a Bak siRNA significantly protected cells from Ad.mda-7–induced apoptosis (Fig. 4E, *, P < 0.05). As DU-145 P a Bak-null cell, we also evaluated Bak and Bak activation using flow cytometry in PC-3 cells, which express both proapoptotic proteins (Supplementary Fig. S1A and B). Infection of PC-3 cells with Ad.5/s.mda-7 resulted in activation of Bak (D ~ 0.21) to a greater extent than Bak (D ~ 0.10).

**RNA interference or gene knockout of Mcl-1 dramatically sensitizes cells to mda-7/IL-24–mediated toxicity**

To further evaluate the role of Mcl-1 in mda-7/IL-24–mediated cytoxicity, both a siRNA strategy and a gene knockout approach were used. DU-145 cells were transiently transfected with a siRNA against Mcl-1 (Fig. 5A, inset). Mcl-1 inhibition dramatically sensitized DU-145 cells to Ad.mda-7 lethality both at high and low doses (50 or 100 pfu/cell; Fig. 5A). Experiments were then performed with Mcl-1−/− and Mcl-1−/− MEFs. As mda-7/IL-24 selectively kills transformed cells, the MEFs were initially transfected with a pcDNA3.1-Ha-ras (vector-overexpressing human Ha-ras); 24 hours later, the cells were infected with Ad.vec or Ad.mda-7. Mcl-1−/− cells exhibited a significant increase in sensitivity to Ad.mda-7 (50 and 100 pfu/cell) compared with Mcl-1−/− cells (P < 0.001; Fig. 5B), presumably by downregulation of Bcl-xL (27). Similarly, Ha-ras–transfected wt MEFs were sensitive to Ad.mda-7. However, Ha-ras–transfected Bax−/−, Bak−/−, and Bax−/−/Bak−/− (double knockout) MEFs displayed significant resistance (*, P < 0.05) to Ad.mda-7 infection (Fig. 5C).

**mda-7/IL-24 downregulates Mcl-1 through inhibition of Mcl-1 translation**

We next analyzed the molecular mechanism of Mcl-1 downregulation by Ad.mda-7. Infection of DU-145 cells with Ad.mda-7 had no significant effect on luciferase driven by a Mcl-1 promoter or total Mcl-1 mRNA level (P > 0.05) even after 48 hours or onward (Fig. 6A, top left and right). In addition, inhibition of transcription using actinomycin D (1 μg/ml) resulted in a decrease in Mcl-1 protein levels, and infection with Ad.mda-7 resulted in a further decline at 12 hours (Fig. 6A, bottom), suggesting an alternative, transcription-independent mechanism of Mcl-1 downregulation by mda-7/IL-24. Immunoprecipitation followed by immunoblot analysis revealed no major changes in Mcl-1 ubiquitination in Ad.mda-7–infected cells even after 48 hours (Fig. 6B, top). Further, Ad.mda-7 infection did not enhance proteasomal activity even after 48 hours (Fig. 6C). Furthermore, blocking the proteasome system with MG132 resulted in a time-dependent accumulation of Mcl-1, which was completely abrogated by concomitant Ad.mda-7 infection (Fig. 6B, bottom left). Moreover, in the absence of MG132, mda-7/IL-24 largely downregulated total Mcl-1 levels, whereas MG132 resulted in a clear increase by opposing proteasomal degradation. Infection with Ad.mda-7 and administration of MG132 resulted in no change in total Mcl-1 levels, indicating that mda-7/IL-24 blocks MG132-mediated Mcl-1 accumulation by preventing new synthesis probably due to inhibition of Mcl-1 translation (Fig. 6B, bottom right). Furthermore, infection with Ad.mda-7 resulted in suppression of eIF4E phosphorylation, which was evident from 18 hours after infection and onward (Fig. 6D). Levels of total eIF4E protein remained unchanged following Ad.mda-7 infection. The levels of both total and phosphorylated eIF4E were increased following infection with Ad.mda-7, whereas phosphorylation of the eIF4E-binding protein-1 declined at later time points. Furthermore, when we analyzed the newly synthesized Mcl-1 in cells pre-labeled with [35S]methionine (pulse chase initiated 16 hours after Ad.vec or Ad.mda-7 infection), in comparison with Ad.vec infection, the stability of Mcl-1 protein in cells labeled with [35S]methionine was not diminished following infection with Ad.mda-7, which indicates that mda-7/IL-24.

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**Figure 3.** Forced expression of Mcl-1 blocks mda-7/IL-24–mediated apoptosis in DU-145 cells in vitro and in vivo. A, DU-145 cells were transiently transfected with pcDNA3.1 or pcDNA3.1-Mcl-1 plasmids, cultured for 20 h, then infected with the indicated pfu/cell of Ad.vec or Ad.mda-7 for 48 h, after which protein lysates were prepared and subjected to Western blotting using the indicated antibody. B, DU-145 cells stably expressing (inset) a transfected Mcl-1 gene (two clones, DU-Mcl-1-8 (1,6-bismaleimidohexane) and DU-Mcl-1-20) or an empty vector (DU-pcDNA3.1) were infected with the indicated pfu/cell of Ad.vec or Ad.mda-7 for 48 h, after which the extent of apoptosis was determined using Annexin V staining assays. Bars, SD (n = 3), P < 0.05 versus DU-pcDNA3.1). C, lysates were prepared from DU-Mcl-1-8 or DU-pcDNA3.1 cells infected with the indicated pfu/cell of Ad.vec or Ad.mda-7 for 48 h, and Western blotting was performed to monitor Mcl-1 and MDA-7/IL-24 protein levels and cleavage of PARP. D, tumor xenografts from DU-Mcl-1-8 and DU-pcDNA3.1 xenograft tumors after 5 wk (inset). F, tumor xenograft models established as indicated in B. Tumors were harvested, paraffin-embedded sections were immunostained for MDA-7/IL-24, and Ki-67 and TUNEL assays were performed using Deadend TUNEL assay kit.

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Figure 4. Forced expression of mda-7/IL-24 promotes Mcl-1 degradation to trigger Bak activation. A, lysates were prepared from DU-Mcl-1-8, a clone of DU-145 cells ectopically expressing Mcl-1, or DU-pcDNA3.1, an empty vector, and infected with the indicated pfu/cell of Ad.vec or Ad.mda-7 for 48 h; expression of Bak was determined by Western blotting. For detection of activated Bak, cell lysates were immunoprecipitated with Bak and Western blotting was performed with anti-Bak Ab-1 that specifically recognizes activated Bak. B, cells were treated as mentioned in A, and Bak activation was detected by flow cytometric analysis of Ad.vec- or Ad.mda-7 (100 pfu/cell for 48 h)-infected DU-Mcl-1-8 and DU-pcDNA3.1 cells stained with anti-Bak Ab-1 antibody. C, DU-Mcl-1-8 and DU-pcDNA3.1 cells were infected with the indicated pfu/cell of Ad.vec or Ad.mda-7 for 48 h, and the mitochondrial fractions were isolated, treated with 1 mmol/L BMH, and then analyzed by SDS-PAGE and immunoblotted with anti-BAK. *, BAK complexes with dimers or trimers. **, inactive BAK conformer. D, DU-Mcl-1-8 (1,6-bismaleimidohexane) and DU-pcDNA3.1 cells were infected with the indicated pfu/cell of Ad.vec and Ad.mda-7 for 24 h in the absence or presence of 25 μmol/L of z-VAD-FMK, after which cells were lysed and subjected to immunoprecipitation using anti-Bak and then immunoblotted with anti–Mcl-1. For comparison, the rightmost lanes (designated as ‘Lysate’) were loaded with whole-cell lysates. E, DU-145 cells were transiently transfected with either control siRNA or Bak siRNA, cells were infected with the 100 pfu/cell of Ad.vec or Ad.mda-7 for 48 h, after which the percentage of apoptosis was determined by flow cytometry after Annexin V–FITC/PI staining. Bars, SD (n = 3; *, P < 0.05).
might block Mcl-1 protein synthesis rather than affect protein turnover (Fig. 6E, top). Finally, we determined Mcl-1 mRNA association with polysomes on infection of DU-145 cells with Ad.vec or Ad.mda-7. After 30 hours of infection, the Ad.mda-7–infected cells exhibited reduced Mcl-1 mRNA binding to polysomes compared with Ad.vec–infected cells (Fig. 6E, bottom).

**Discussion**

Among the members of this Bcl-2 gene family, Mcl-1 plays a central role in prostate carcinogenesis (36). For example, a study examining 64 cases of prostate adenocarcinoma with different Gleason scores and 24 cases of prostatic intraepithelial neoplasia (PIN) found that the percentage of...
Mcl-1–positive cells was higher in tumors with Gleason grades 8 to 10 than in PIN or lower-grade tumors (23). These data are consistent with the results of our study; however, we did not observe any correlation between increased expression of Mcl-1 and stages of the disease. These results indicate that Mcl-1 might be more essential for tumor cell survival in the initial stages of carcinogenesis rather than playing any direct role in cancer progression.

Elimination of Mcl-1 has been shown to be required for apoptosis induction in response to a number of ER stimuli, including thapsigargin (22, 31) and sorafenib (31). Downregulation of Mcl-1 in ER-stressed cells is a consequence of stress-induced phosphorylation of eIF2α at Ser51 (22). This interpretation is supported by the observations that when DU-145 cells were transfected with a dominant-negative eIF-2α or a dominant-negative PERK, mda-7/IL-24–mediated induction of apoptosis and Mcl-1 downregulation were inhibited. It should be noted that PERK activation is generally considered a marker for ER stress and UPR, and necessary for activation of eIF2α (37). PERK is an ER-resident type I transmembrane protein whose NH2-terminal luminal domain is sensitive to the upstream ER stress signal and whose COOH-terminal cytoplasmic domain directly phosphorylates eIF2α. Under conditions of ER stress, BiP/GRP78 increases the load of ER client proteins, whereas loss of BiP/GRP78 binding correlates with oligomerization, trans-autophosphorylation, and activation of downstream signaling by PERK (37, 38). It is worth noting that in GBM cells and transformed fibroblasts, MDA-7/IL-24–activated PERK and PERK−/− transformed fibroblasts or GBM cells expressing a dominant-negative PERK

Figure 7. Mcl-1 is an important regulator of mda-7/IL-24–mediated apoptosis. A, DU-145 cells were infected with 100 pfu/cell of Ad.vec or Ad.mda-7, and protein lysates were prepared at the specified times and subjected to Western blotting using indicated antibodies. B, model for mda-7/IL-24 lethality through induction of an ER stress response and Mcl-1 downregulation.
were resistant to MDA-7/IL-24–induced killing. Furthermore, overexpression of the PERK chaperone BiP/GRP78 blocked mda-7/IL-24–induced PERK activation and cell killing (29). BiP/GRP78 interacts physically with MDA-7/IL-24 in prostate cancer cells, and hence is considered a primary target for MDA-7/IL-24 in these cells (18).

Our findings rule out a significant role for altered Mcl-1 transcription, mRNAs stability, or enhanced proteasomal degradation activity of Mcl-1 when DU-145 cells were infected with Ad.mda-7. The polysomal association of Mcl-1 mRNA provided evidence of marked inhibition of Mcl-1 translation following infection with Ad.mda-7. Diminished Mcl-1 translation has been implicated in Mcl-1 downregulation in several systems, including HeLa cells exposed to UV (39) and BAY 43-9006–mediated lethality in both U937 and K562 cells (30). Translation of most mRNAs is dependent on the cap structure m7-GTP, which is found at the 5′ terminus of all cellular eukaryotic mRNAs. The cap-binding protein eIF4E represents the rate-limiting member of the eIF4F complex and binds directly to the 5′-terminal m7-GTP cap, resulting in recruitment of ribosomes to the 5′-end of the mRNA transcripts (40). Infection with Ad.mda-7 results in a marked decrease of eIF4E protein phosphorylation, and an increase in both total and phosphorylated eIF4G, which could be a possible reason for reduced polysomal binding of Mcl-1 mRNA.

In summary, mda-7/IL-24 induces transformed cell-selective antitumor activity through ER stress by inhibiting the translation of Mcl-1 protein in prostate cancer, which leads to activation of Bak. Earlier, we reported the importance of Bcl-Xi in protecting against mda-7/IL-24 lethality (27). Both Bcl-Xi and Mcl-1 can sequester Bak (35); however, it is important to note that ectopic expression of mda-7/IL-24 can moderately downregulate Bcl-Xi while, at the same time point, Mcl-1 disappeared completely (Fig. 7A). The functional redundancy of these two antiapoptotic proteins depends on the fact that only one of them is tightly regulated by the therapeutic treatment of mda-7/IL-24 (Fig. 7B). Overall, our present study highlights the possibility that mda-7/IL-24 may cooperate synergistically with Mcl-1 small-molecule inhibitors to induce cancer cell death, which is an area for future investigation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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